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ANTIMICROBIAL ACTIVITY OF ADHATODA VASICA FLOWERS

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ABSTRACT

Adhatoda vasica belonging to Acanthaceae family, commonly known as Adosa, is found in many regions of India and throughout the world, with a multitude of uses in traditional Ayurveda. Vasica is most well-known for its effectiveness in treating respiratory conditions and are highly demandable in pharmaceutical industries. The compound isolated from ethyl acetate fraction of Adhatoda vasica flowers extract has a significant antibacterial activity against bacteria and fungi.Four bacterial strains like S. typhi, E. coli, E. faecalis and B. cereus and two fungal strains such as C.lunata and C.albicans were tested by using disc diffusion method. The anti bacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with the standard Chloramphenicol and the anti-fungal activity of the compound isolated from ethyl acetate fraction is almost comparable with the standard Fluconazole. These are the drugs from Adhatoda vasica flowers that either destroy or inhibit the growth of bacteria and fungi. It has also the ability to prevent or treat bacterial and fungal infections. Further studies are highly needed for future drug development. The present research aims to compile medicinal values of Adhatoda vasica flowers generated through the research activity using modern scientific approaches and innovative scientific tools.

KEY WORDS

Adhatoda vasica Flowers, Antibacterial activity, Antifungal activity, Diffusion method, Chloramphenicol, Fluconazole etc.

INTRODUCTION

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. The status of herbal medicine has been fast growing all over the world during the last few decades[1]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants[2]. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources[3]. The ancient man discovered the therapeutic value of some herbs by trial and error. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils[4]. Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains. Plants have a great potential for producing new drugs of great benefit to mankind[5]. Medicinal plants represent a rich source from which antimicrobial agents may be obtained[6].

Adhatoda vasica, an important Indian medicinal plant has long been used in ayurvedic system of medicine. The plant has been found to diverse number of pharmacological activities include Respiratory tract infection, cough formulation, expectorant, anti-spasmodic and bleeding pills[7]. Recently various researchers have found greater interest in anti-microbial activity against several species in different studies. *Adhatoda vasica* (Family *Acanthaceae*) is commonly known as Malabar Nut, is distributed throughout India up to an altitude of 1300m. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping

cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic in traditional medicines. It was also used by traditional midwives at the time of delivery [8]. The plant has been reported to have high medicinal value[9].

The development of resistance in the microorganisms due to the use of antibiotics has created an emergency to unravel the antibacterial compounds from the plants. A medicinal plant is any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or as a chemo pharmaceutical semi synthesis. In order to improve the efficiency and ethics of modern medical practice, researchers are increasingly turning their attention to folklore medicines as a source of new drugs (Swiader et al., 1997)[10]. Bioactivity of essential oils of temperate aromatic plants were revealing antibacterial, antioxidant, anti-inflammatory and other related pharmacological activities(Svoboda et al., 1998)[11].The antiseptics qualities of aromatic and medicinal plants and their extracts have been recognized antiquity, several attempts to characterize these properties in the laboratory data back (Martindale, 1910)[12].

The plant volatile oils were used for their antibacterial properties against 25 genera of bacteria,using agar diffusion technique(Deans et al.,1987).[13] In the present study, we have chosen Adhatoda vasica as herbal medicine to determine their antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the invitro antimicrobial activity of ethyl acetate extracts of this plant against some Gram-positive and Gram-negative pathogenic microorganisms that causes the most common cases of infectious disease.[14] Antimicrobials have the significant clinical value in the treatment of resistant microbial strains. Therefore in the present study, Adhatoda vasica was screened for their antimicrobial activity.

MATERIALS AND METHOD

Extraction and fractionation

Fresh flowers (1kg) of Adhatoda vasica were collected at O.Koothur village, Ariyalur district, during the month of August and identified by Dr.John Britto, Director, Rabinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Trichirappalli-2, Tamilnadu. India. The flowers were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction was taken for screening anti-microbial activities.

Antimicrobial Procedure

Screening of Antibacterial Activity

Bacteria tested

Four bacterial strains such as S. typhi, E. coli, E. faecalis and B. cereus were used throughout this investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums

Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that was incubated without agitation for 24 hrs at 37^{o}C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0x10^6 colony forming units (CFU/ml).

Antibacterial susceptibility test

The disc diffusion method was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The test sample of concentration 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic Chloramphenicol of concentration 1mg/ml was used as positive control.

Screening of Antifungal Activity

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hrs and the suspensions were checked to provide approximately 105 CFU/ml.

Determination of antifungal activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution. Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37ºC for 72 hrs. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION

In the current study, the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers exhibited significant antimicrobial activity when compared with standard drug. It is clear from the data presented in Table I that the sample possesses antibacterial activity. The disc diffusion method results showed the zone of inhibition for 10 mg/ml as 7 mm, 6 mm , 7 mm and 7 mm, for 20 mg/ml as 9 mm,9mm , 10 mm and 9 mm, for 30 mg/ml showing 12 mm, 12 mm , 12 mm and 11 mm and for 40 mg/ml as 17 mm, 15 mm , 16 mm and 16 mm, against S. typhi, E. coli, E. faecalis and B.cereus respectively when compared with standard drug Chloramphenicol showing 19mm, 21 mm , 22 mm and 21 mm zone of inhibition respectively. Then it is clear from the data presented in Table II that the sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 7 mm and 6 mm, for 20 mg/ml as 8 mm and 9 mm, for 30 mg/ml as 12 mm and 11 mm and for 40 mg/ml as 16 mm and 15 mm against C.lunata, and C.albicans respectively when compared with standard drug Fluconazole showing 21mm and 19 mm of inhibition respectively. The above result shows that the activity of isolated ethyl acetate fraction of Adhatoda vasica flowers shows considerable antibacterial and antifungal activities and also the possession of antimicrobial activities against a number of microorganisms.

S.No.	Name Of Organisms	Zone of inhibition(mm)				
		Standard (Chloramphenicol)	Sample Concentration (mg/ml)			
			10	20	30	40
1.	S.typhi	19	7	9	12	17
2.	E.coli	21	6	9	12	15
3.	E.faecalis	22	7	10	12	16
4.	B.cereus	21	7	9	11	16

Table 1: Antibacterial activity of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers in different strains

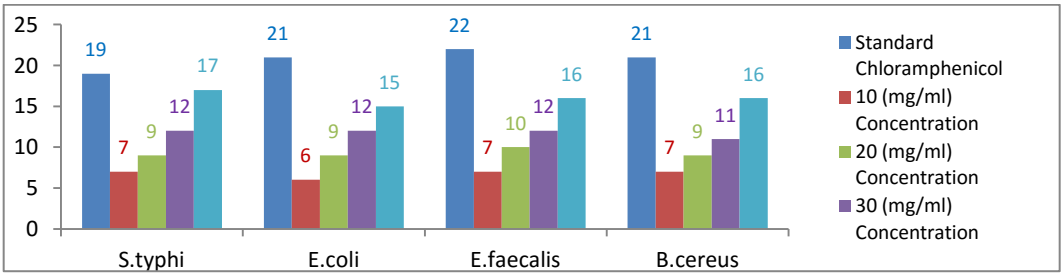
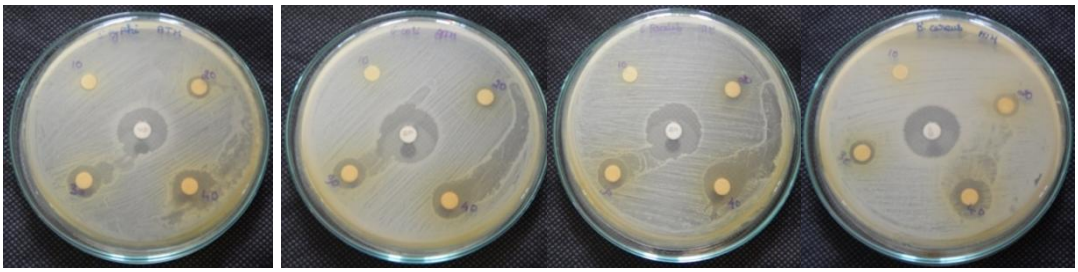


Figure.1: Graphical representation of anti bacterial activity of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers. (Standard: Chloramphenicol, concentration 1 mg/ml).



S.typhi **E.coli** **E.faecalis** **B.cereus**

Figure 2: Inhibition of bacterial growth of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers by Disc diffusion method.

S.No.	Name Of Organisms	Zone of inhibition(mm) Standard (Fluconazole)	Sample Concentration (mg/ml) 10	20	30	40
1.	C.lunata	21	7	8	12	16
2.	C.albicans	19	6	9	11	15

Table: 2. Anti fungal activity of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers in different strains

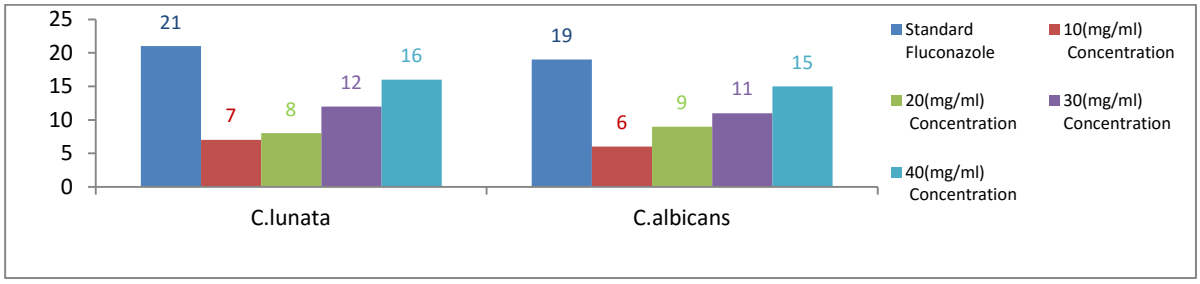
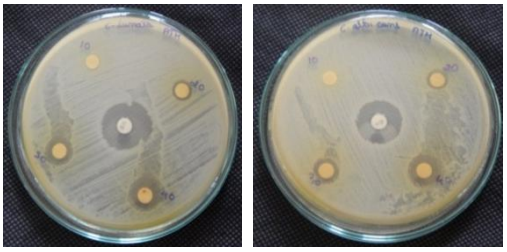


Figure 3: Graphical representations of anti fungal activity of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers. (Standard: Fluconazole , concentration 1 mg/ml)



C.lunata **C.albicans**

Figure 4: Inhibition of bacterial growth of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers by Disc diffusion method.

CONCLUSION

Based on the result of the above study on the Adhatoda vasica we conclude that Adhatoda vasica shows better antibacterial and antifungal activity against following microorganisms like (Bacterias)S.typhi, E.coli , E.faecalis B.cereus and (fungal) C.lunata C.albicans. Also it justifies the claimed uses of flower parts of the Adhatoda vasica in the traditional system of medicine to care for various infectious disease caused by the microbes. Antimicrobial activities are motivated by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, It is necessary to isolate and characterize the active

compounds. Moreover, extract of the plant should be investigated for better understanding of its safety, efficacy and properties.

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